

NTP Taps Disinfection By-Products for Study



The use of chlorination to purify water supplies is considered one of the most important public health advances of the twentieth century. Following the 1908 introduction of widespread water chlorination, once-common diseases such as cholera, dysentery, and typhoid fever were practically eliminated. However, the chlorination cure-all proved to have a caveat: disinfection by-products (DBPs), which result from the reaction between the chlorine added during chlorination and organic material such as leaves and sediment in the source water. In the mid-1970s, certain DBPs were found to cause adverse health effects including cancer in laboratory animals.

In November 1992, in accordance with the Safe Drinking Water Act, the U.S. Environmental Protection Agency (EPA) convened a committee consisting of representatives from state and local regulatory and public health agencies, local government, consumer groups, public water systems, environmental groups, and the EPA itself to address the issue of regulating DBPs in drinking water. The committee decided that more information was needed on DBPs before final legislation could be written, so a two-stage rule was enacted.

The stage 1 DBP rule, intended as an interim rule to get some framework in place, was promulgated in December 1998. Based on the best available science, the rule set maximum contaminant level goals or maximum residual disinfectant level goals for over 20 DBPs and DBP mixtures. The final stage 2 DBP rule is due by May 2002. There are, however, still insufficient data for scientists and legislators to establish DBP standards with confidence.

To fill in the missing data, the EPA's Office of Water and National Health and Environmental Effects Research Laboratory (NHEERL) established a research program for mechanistic research on DBPs and turned to the National Toxicology Program (NTP) for its expertise in evaluating environmental chemicals. Together, the EPA and the NTP are systematically evaluating several priority DBPs in order to give policy makers the information they need to set sound drinking water standards.

The DBP Dilemma

The most commonly occurring DBPs are the trihalomethanes (THMs) and the

haloacetic acids (HAAs). Other chlorination DBPs include a family of chemicals called the haloacetonitriles and a compound known as mutagen X (MX).

In 1976 the National Cancer Institute issued its *Report on the Carcinogenic Bioassay of Chloroform*, which stated that chloroform, the THM found most commonly and in the greatest concentrations in drinking water, causes cancer in rodents. Since then, additional studies have shown that other chlorination DBPs such as bromodichloromethane, chlorodibromomethane, bromoform, and dichloroacetic acid also cause cancer in rodents. A study by Kenneth P. Cantor and colleagues at the National Cancer Institute, published in the December 1987 issue of the *Journal of the National Cancer Institute*, found that people who drank 8 cups of chlorinated tap water for 40–59 years had a 40% greater risk of bladder cancer than those who drank less tap water or unchlorinated water. People who drank 8 cups of chlorinated tap water for 60 or more years had an 80% greater risk of bladder cancer. Although cigarette smoking and occupational exposure to carcinogens remain the primary causes of bladder cancer, the study scientists said, THMs in drinking water cannot be discounted as a contributing factor.

Chlorination isn't the only disinfection process that yields DBPs, however. Alternatives to chlorine disinfection—which include the use of chlorine dioxide, ozone, and chloramination—produce their own by-products, many of which can cause adverse health effects in rodents and, possibly, humans. The addition of chlorine dioxide to drinking water creates sodium chlorate. (Currently, there are few toxicity data on sodium chlorate.) Treatment with

ozone causes few DBPs but does result in the production of bromate when used in water with high bromine concentrations. Bromate has been shown in studies by the EPA to cause mesotheliomas in rats and kidney tumors in both rats and mice. In addition, oral exposures to potassium bromate have been shown to cause renal cancer in rats. Finally, chloramination, the addition of both chlorine and ammonia to drinking water, reduces the incidence of THMs

but produces chloramines, which in high doses cause blood and liver damage in laboratory animals. The effects of chloramines on humans are currently unknown.

According to Gary Boorman, associate director of the NTP Office of Special Programs and coordinator of DBP studies at the NIEHS, only one-third of people's exposure to DBPs comes through drinking water (including its use with coffee, tea, and foods such as juice concentrates and instant soups). People are also exposed dermally and inhalationally through activities such as cooking, bathing, swimming in pools, and using dishwashers and humidifiers. Because it is difficult to avoid DBP exposure, it is crucial that scientists

understand the effects of these chemicals. However, it's hard to accurately measure how much DBP one is exposed to because the occurrence and concentration of a particular by-product is highly dependent on the method of disinfection used and other variables such as the amount of organic matter in the water, the temperature of the water, and the distance the water is piped from the site of disinfection.

DBP Studies

For each compound studied, the NTP collaborators are examining some combination



of carcinogenicity, reproductive toxicity, neurotoxicity, and immunotoxicity, with molecular biology studies to follow up in selected areas. The NTP is using both traditional rodent models and transgenic mice to conduct long- and short-term toxicity studies. In addition, program scientists led by James Burkhart, chief of the Alternative Test Systems Group in the Laboratory of Toxicology, are working with the Department of Defense under the supervision of Hank Gardner, scientific director of the U.S. Army Center for Environmental Health Research at Fort Detrick, Maryland, to evaluate the effects of low doses of chloroform, chlorate, and bromodichloromethane in the Japanese medaka (*Oryzias latipes*), a small fish model. Because standard rodent models are not always responsive to every DBP, especially at low concentrations, study planners hope the transgenic models and the medaka can help determine the effects and toxicity of individual DBPs and DBP mixtures at low concentrations.

Chemistry. The chemistry of the DBPs is complex, especially at low concentrations. Chemistry Resources Group leader Cynthia Smith is responsible for ensuring that in all NTP studies the animals are exposed to DBP concentrations according to each study design. This includes procuring the purest available material, characterizing the purity and identity of each DBP, developing reproducible techniques for formulation and chemical analysis, and verifying their stability in tap water. Most of the chemicals were available commercially, but bromodichloroacetic acid and MX were synthesized for these studies.

In collaboration with the NTP, scientists from the NIEHS, the EPA, and the Chemical Industry Institute of Toxicology (CIIT) are conducting studies to determine the blood and tissue levels of DBPs in both humans and rodents following drinking water, dermal, and inhalation exposures. In these toxicokinetic studies, human volunteers are exposed at concentrations typically found in drinking water while the rodents are exposed at much higher concentrations. Toxicokinetic studies will be useful in extrapolating the results from rodent studies to estimate possible hazards for humans exposed at the much lower concentrations found in drinking water.

Carcinogenicity. Long-term rodent carcinogenicity studies are being developed for six compounds under Boorman's direction and one compound (MX) under the direction of Ronald A. Herbert of the Pathology Support Group in the Laboratory of Experimental Pathology. The studies will focus on low-dose exposures to the various compounds. Boorman explains that doses of

DBPs found to cause cancer in rodents in past studies were as much as 200,000–400,000 times higher than the concentrations typically found in drinking water, so it is important to characterize the effects of the concentrations that people realistically are more likely to encounter.

Bromodichloromethane, a THM, had been found to cause colon, kidney, and liver tumors in rodents in previous NTP studies. Further NTP studies will look at the effects of lower doses on the intestines, kidneys, and liver in rats and mice, transgenic mice, and the medaka. NTP researcher June Dunnick is also evaluating bromodichloromethane in a transgenic mouse model with a mutated APC tumor suppressor gene that is specifically sensitive to the development of intestinal cancer. Because bromodichloromethane exposure may also occur by inhalation, the NIEHS is collaborating with the CIIT on bromodichloromethane inhalation studies using transgenic mice.

Dibromoacetic acid, an HAA, was selected for study because it occurs frequently and there are far fewer toxicology data on this compound than on other HAAs. Based on evidence that kindred compounds trichloroacetic acid and dichloroacetic acid (which is also being studied further) cause liver cancer in rodents at 0.5 grams per liter and higher, scientists expect that liver tumors may be associated with exposure to high concentrations of dibromoacetic acid as well. Studies are also being planned for bromodichloroacetic acid, one of the brominated HAAs, which (based on preliminary data) are suspected of being more toxic than their chlorinated HAA sisters. There is practically no information on the haloacetonitrile family of DBPs, so dibromoacetonitrile was chosen for study as a representative for the group. Based on the results of this evaluation, other compounds in the family may be tested later.

Chlorate, produced by the alternative disinfectant chlorine dioxide, has not been extensively tested for toxicity data, but it is known that high doses of chlorate can cause methemoglobinemia ("blue baby syndrome") and blood cell destruction. Preliminary evidence by the Department of Defense suggests that the medaka may be quite insensitive to chlorate, while NTP and EPA studies suggest that rats but not mice develop thyroid follicular cell hyperplasia when exposed to high chlorate concentrations. Studies to determine the mechanistic underpinnings for this hyperplasia are underway in a collaborative effort between NIEHS and EPA scientists. Bromate, produced during ozonation and shown to cause cancer in standard rodent studies, is being tested extensively. The NTP will study the effects of sodium bromate in

transgenic mouse models and, depending on the results, further studies may be planned.

MX, which has been shown to cause thyroid and liver tumors in rats, is being considered for studies in both rats and mice. Although it occurs at relatively low concentrations in drinking water, MX is believed to cause as much as 50% of the mutagenicity associated with chlorinated tap water.

Reproductive toxicity. Robert Chapin, a reproductive toxicologist in the Laboratory of Toxicology, is responsible for studies on 10 compounds identified as high priorities by the EPA: the THMs bromodichloromethane and chlorodibromomethane; the HAAs bromochloroacetic acid, dibromochloroacetic acid, and tribromoacetic acid; the haloacetonitriles bromoacetonitrile and dibromoacetonitrile; hexachloropropanone (a member of a lesser class of DBPs called the haloketones); sodium bromate; and a mixture of mono-, di-, and trichloroacetic acids, mono- and dibromoacetic acids, and bromochloroacetic acid (all HAAs).

Chapin has completed studies on the nine individual compounds using a short-term (21-day) screen that allows researchers to test for a wide range of reproductive, developmental, and general toxicity end points in parallel groups rather than serially, as they normally would be. According to Chapin, the first nine studies did not reveal any striking reproductive or general toxicities. "There were palatability problems long before there were significant toxicities," he says, meaning that the chemicals made the water taste too bad to drink before any toxic concentrations were reached. The team is now down to the last test, the big mixture. Chapin says of the reproductive toxicity tests, "They don't cover effects on animals once they've grown up, which is to say, what we were primarily interested in were the effects on the parents—can they make babies successfully and do those babies seem healthy? The whole endocrine disruptor issue has refocused our attention on the question of 'if a kid looks healthy, will it still be healthy when it grows up—can it find a mate? Can it ward off infections?'" In future studies, Chapin and colleagues will be looking not just at reproductive toxicity but also neurotoxicity and immunotoxicity all in one cohort of animals exposed either during development or during gestation and development.

Neurotoxicity. Recently, Ginger Moser and colleagues at the NHEERL studied the neurotoxicity of dichloroacetic acid and published their results in the November–December 1999 issue of *Neurotoxicology and Teratology*. They found that exposure to very low doses of dichloroacetic acid—as low as 16 milligrams per kilogram of body

weight per day—caused progressive neuropathy in the limbs (primarily the hind limbs) of rats. The neuropathy manifested as limb weakness, altered gait and righting reflex, reduced grip strength, tremors, and an unusual clasp of the front paws to the chest. The rats also experienced ocular abnormalities including loss of pupil reflex. Although earlier studies had noted neurotoxic effects in rats upon exposure to dichloroacetic acid, the EPA/NTP study was the first to show these effects at such low doses.

Based on the strength of those findings, Moser and colleagues at the NTP decided to study additional DBPs. Currently, they are studying the neurotoxicity of dibromoacetic acid, and Moser expects these studies to yield interesting results. She says, “Dibromoacetic acid is very similar to dichloroacetic acid chemically; reproductive studies show that it affects sperm even more strongly than dichloroacetic acid does.” Collaborative studies on bromodichloromethane and dibromoacetonitrile are also in the works. To Moser’s knowledge, no one has ever studied the neurotoxicity of DBPs other than dichloroacetic acid.

Jean Harry, chief of the Neurotoxicity Group in the Laboratory of Toxicology, is coordinating the efforts between the EPA and the NIEHS. “We’re choosing chemicals to study based on their interest in neurotoxicity,” she says. She emphasizes that the studies are meant to evaluate subtle effects that may manifest. “We’re not expecting any particular type of effect; we’re doing a broad screen for whatever neurobehavioral end points may be affected,” she says. The studies will use the Functional Observational Battery (FOB), the standard multinationally validated testing battery recommended by the EPA for chemical and pesticide screening.

Immunotoxicity. Dori Germolec is chief of the Immunotoxicity Group in the Laboratory of Toxicology. She oversees a contract with Virginia Commonwealth University in Richmond in which dibromoacetic acid, dichloroacetic acid, sodium bromate, and sodium chlorite (used during treatment with chlorine dioxide) are being tested for immunologic effects. These compounds were selected for study by the Office of Water based on historical reports that they exhibit some immunologic effects. Germolec’s group will also be studying chloramine and chloroform.

Each of the first four compounds has undergone a screening study in which widely spaced dose levels were used to identify whether there appeared to be an immune target. Identification of an immune target

would qualify a compound for a full protocol study to further identify the specific cell types or arm of the immune system targeted by the compound, says Germolec. She says that sodium bromate and sodium chlorite showed no immunologic effects and will undergo no further testing. Dichloroacetic acid exhibited weak, statistically insignificant effects.

Dibromoacetic acid, however, was shown to cause significant immune effects. When administered in drinking water at doses of 125–2,000 milligrams per liter, the compound induced dose-dependent effects including changes in organ weights, blood count parameters, and numbers of antibody-producing spleen cells. The most marked effect was observed in the 500-milligram-per-liter dose group, which experienced a 20% increase in spleen cell number compared to control animals. Based on the results of this preliminary study, a full protocol study of dibromoacetic acid has been launched and is currently in progress.

Molecular biology. Robert Sills, chief of the Molecular Pathology Group in the Laboratory of Experimental Pathology, and Harry will pick up where the neurotoxicity and carcinogenicity studies leave off. Specifically, their work will provide mechanistic data for characterizing the toxicity of carcinogenic DPBs at the molecular level.

For compounds that induce a tumor response, Sills will examine genetic alterations in the rodent tumors. “An understanding of the mechanism of the toxicity and of carcinogenicity will allow for a better understanding of the relevance of animal data to humans,” he says. He explains that genes altered in human tumors have counterparts in the rodent genome that are likely to be the genes affected in rodent tumors. By identifying whether the rodent gene alterations correspond with known human cancer mechanisms, it may be possible to better estimate whether a compound is likely to also cause tumors in humans.

In following up on the neurotoxicity evaluations, Sills and Harry will be looking at components of both the peripheral and central nervous systems related to effects that may be seen in the neurobehavioral screening battery conducted by Moser. For instance, in the dichloroacetic acid studies in which rats suffered hind limb weakness, they will study the parts of the nervous system that innervate the hind limbs including the sciatic nerve, associated motor system areas in the spinal cord, and the cerebellar region of the brain in order to identify cellular changes. “We will look histopathologically at areas that are identified as suspicious in the FOB,” says Harry.

A Complex Issue

Today, the EPA is seeking to minimize the potential health effects due to DBPs while still protecting the public water supply against microbial pathogens. Because DBP production depends so heavily on the physical characteristics of the water itself, Boorman says water utility companies can help reduce DBPs by changing the pH and other characteristics of the water during the chlorination process. One planned benefit of the ongoing research on DBPs is an ability to advise water utilities on altering their techniques without compromising water quality.

One of the primary issues in DBP evaluation is the need to balance risks and benefits—the risks of DBPs versus the risks of nonchlorinated water. Boorman cites the example of a cholera epidemic that began in 1991 in Chancay, Peru, after authorities decided to discontinue water chlorination following reports that DBPs presented a cancer risk to humans. By the end of 1992, the epidemic had spread throughout Latin America, and Peru alone suffered some 350,000 cases. (Many Peruvian localities have since resumed water chlorination.) Although the EPA stated in 1992 that there was no demonstrable link between DBPs and cancer in humans, concerns remain today.

A new concern about DBPs is that as water temperatures rise in response to global warming, the amount of organic material in the water also rises—and the more organic material there is in the water, the more DBPs can be produced. Boorman says there has been a reported increase in blue-green algal blooms. He wants to find out how prevalent the blooms are and study the effects of blue-green algae toxins (microcystins) on water quality.

Boorman also notes that humans are more often exposed to complex mixtures of DBPs and other water contaminants at very low concentrations than to single chemicals. In addition, most of the data available are for chlorination by-products because this has traditionally been the water disinfection method of choice in the United States, although alternative disinfectants are being used more widely. In order to make sound policy decisions regarding water treatment, it is necessary to have more information on the relative risks of the different disinfection processes as well as information on the extent of exposures to DBP mixtures—information that is not yet available.

Drinking water safety is a complex issue, says Boorman. “Drinking water issues are so big and complex that no one agency has the resources and information to address them,” he says. “It’s in everyone’s best interest to work together.” —Susan M. Booker



Evaluating Floyd's Effect on Health in Eastern North Carolina

Since the now-famous storm of September 1999, the name "Floyd" has taken on new associations for many people in eastern North Carolina: devastation and environmental catastrophe. On 12 November 1999, representatives from state and federal agencies and local universities met on the NIEHS campus in Research Triangle Park, North Carolina, to discuss the environmental health implications of the floods following Hurricane Floyd. In addition to determining the potential long-term human health and ecological problems, participants hoped to develop strategies, collaborations, and partnerships to be used in the restoration of eastern North Carolina.

The meeting served as a forum for sharing information and comparing notes on the problems posed by Floyd from people who are closely involved in current remediation and public health efforts. Moderators led discussions on the overall poststorm status of eastern North Carolina. Roundtable discussions focused on specific environmental concerns such as contaminated water supplies, animal waste spills, disturbed water and marine ecosystems, and the public health response to the storm. Finally, possible future disease outcomes, including infectious and respiratory disease, and prospects for building partnerships for research were discussed.

Many participants felt the most egregious long-term health effects will prove to be those related to the psychological stress associated with the storm. Participants acknowledged that many health effects attributable to the storm will probably be buried under the anonymity of chronic illness (such as preexisting asthma) or perceived mundanity (such as back strain from cleaning up debris). It was also acknowledged that it may be too early to distinguish the full health impact or other long-term ecological and environmental effects of the storm. For example, it is not known if contaminants were introduced into estuary sediments that may have future effects on bottom-dwelling sea creatures and higher organisms in the food chain.

A lack of baseline health data among the populations of eastern North Carolina makes it difficult to accurately gauge Floyd's environmental health effects. Many problems that have rocketed into public awareness, such as asthma and well-water contamination, may have existed before Floyd hit. Still, these issues are important as residents return to homes and work environments that may pose medium- to long-term public health hazards.

While some parts of the puzzle are missing, there are plenty of pertinent data that can be drawn into remediation and public health protection efforts, said attendees. For example, the U.S. Environmental Protection Agency, the U.S. Geological Survey, and the North Carolina Department of Environment and Natural Resources have conducted monitoring of waterways. According to meeting attendees, these data need to be consolidated into a cohesive body of knowledge, perhaps pooled into one database to be used for evaluation and prediction of environmental outcomes.

Immediately following the hurricane, the North Carolina Department of Health and Human Services initiated surveillance of 150 reportable conditions diagnosed in 18 regional hospitals. These cases could establish a cohort for collaborative research between the state, federal research agencies, and academic researchers. Analysis of research conducted after other natural disasters may also be helpful in responding to the floods.

Participants agreed that education is the key to public health protection. Many reasoned that health policies are driven by public opinion and that the public and legislators must therefore be educated on the relative risks of the various problems presented by Floyd. Based on an informed understanding, wise remediation decisions can be made and sound policies for rebuilding and future growth can be established. In addition, long-term programs evaluating the human health and environmental effects of this disaster will help minimize effects of future events.

A report summarizing the public health and environmental issues identified at the meeting will be published in a future issue of *EHP*.
—Susan M. Booker